Severe Combined Immunodeficiency (SCID) in the Mouse

Pathology, Reconstitution, Neoplasms

R. PHILIP CUSTER, MD, GAYLE C. BOSMA, MS, and MELVIN J. BOSMA, PhD

From the Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania

Histologic findings in mice with severe combined immunodeficiency (SCID) were remarkably uniform, consisting of lymphopenia, a rudimentary thymic medulla without cortex, relatively empty splenic follicles and lymph nodes, and undeveloped bronchial and gastrointestinal lymphocytic foci. Fluorescence-activated cell sorter studies revealed a few T cells (apparently nonfunctional) in thymus and spleen; interestingly, these cells seemed highly disposed to neoplasia, because thymic T-cell lymphomas were observed in 41 of 269 mice. No pre-B or B cells could be identified. Cells of the myeloid lineage appeared normal. Reconstitution of lymphoid tissues was achieved after intravenous injection of histocompatible bone marrow cells. (Am J Pathol 1985, 120:464–477)

THERE ARE many distinct human syndromes of immune deficiency. Some are congenital,[1,2] others are naturally acquired,[1-3] and still others are iatrogenically induced.[4] Affected individuals show increased susceptibility to infections, often fatal, and may also show a propensity for the development of lymphoid malignancies.

Given the heterogeneity of immune deficiency syndromes in humans and the limitations imposed on experimentation, a simple animal model for impaired immunity would be advantageous for studying the relationship between immunity and disease, as well as lymphoid differentiation of hematopoietic stem cells. We recently reported such a model in the mouse,[5] namely, a mutant in which the differentiation of both T and B lymphocytes is severely impaired. Mice homozygous for the mutant gene lack functional T and B lymphocytes[5,6] and consequently show severe combined immunodeficiency (SCID). Hence, we denote the mutation as *scid* and affected mice as scid mice. Scid mice have little or no immunity and readily succumb to infections by one or more microorganisms (eg, viruses, bacteria, protozoa, fungi). To ensure survival, they must be raised in a protected environment free of pathogenic agents. Even in such an environment scid mice are prone to premature death, due in part to a high incidence of spontaneous lymphomas which appear to arise from thymic T cells.

In our initial report,[5] the conspicuous histologic features of the scid mouse were illustrated.* The present report details further cytologic observations and includes studies on the lymphoid tissues from scid mice reconstituted with normal bone marrow. Histologic and serologic cell surface analyses of the spontaneous lymphomas in scid mice are also presented.

Materials and Methods

Mice

The *scid* mutation occurred in the C·B-17 inbred mouse strain. C·B-17 is a BALB/c strain that carries the immunoglobulin heavy-chain locus (*Igh*) of the C57BL/6Ka mouse strain.[7] C·B-17 mice homozygous and heterozygous for *scid* are here simply denoted as scid and scid/+ mice, respectively. C·B-17 (Igh^b), BALB/c (Igh^a), (B6·C-9 × BALB/c)F_1 (Igh^a) and scid/+ mice served as normal controls and/or as a source of normal bone marrow for the reconstitution of scid mice (B6·C-9 is an Igh^a congenic partner strain of C57BL/6[7]).

All mice were produced by specific pathogen-free (SPF) breeders in the "barrier facility" of this institute.

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Address reprint requests to Dr. R. Philip Custer, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111.

Scid mice were housed in microisolator cages (Lab Products, Maywood, NJ) containing autoclaved food, water, and bedding; cages were changed in a Class II type safety cabinet (Bellco Glass, Vineland, NJ).

Cell Transfer for Reconstitution

Scid mice were given intravenous injections of 3–5 × 10^6 bone marrow cells from normal C·B-17 (Igh^b), BALB/c (Igh^a), or (B6·C-9 × BALB/c)F_1 (Igh^a) mice. Recipients underwent necropsy 5–10 months after the cell transfer. Successful engraftment of donor cells was apparent from the expression of donor Igh^a allotype in all applicable cases. In order to visualize thymic macrophages and evaluate the putative "blood–thymus barrier,"[8] reconstituted scid mice and scid/+ controls were given intravenous injections of 0.2 ml of a 1:10 dilution of Pelican India Ink in Hanks' balanced salt solution (HBSS) that had been strained through Nitex (HC-3-48 mesh) (Teco, Inc., Elmsford, NY).

Fluorescence-Activated Cell Sorter (FACS) Analysis

Approximately 10^6 cells from thymus, bone marrow, and spleen of scid and scid/+ mice were incubated with various antibody preparations for 45 minutes at 4 C in 0.1 ml of HBSS (without phenol red), supplemented with 0.1% bovine serum albumin and 0.1% NaN3. The antibody preparations included monoclonal antibodies specific for pre-B and B-cell determinants (rat 2C2[9] and 6B2[10] anti-Ly-5 [B220]); monoclonal antibodies[11] reactive with T cells (rat anti-Ly-1 [53.7] and anti-Ly-2 [53.6]); fluorescein isothiocyanate (FITC)-conjugated rat monoclonal (30-H12) to Thy-1.2[11]; FITC-conjugated rabbit antiserum to rat Ig; and FITC-conjugated goat antiserum to mouse IgGl. The last two FITC-conjugated antisera were used as second-stage reagents. Cells were washed twice in HBSS after each incubation, counterstained with 10 μl of ethidium bromide (100 μg/ml) for 10 minutes at room temperature, and analyzed on a fluorescence-activated cell sorter (FACS II, Becton-Dickinson, Mountain View, Calif). Cells were analyzed on the basis of size and fluorescence. Dead cells, ie, those stained with ethidium bromide, were excluded from analysis.

Histologic Preparations

In the present study 353 mice underwent necropsy, including 269 scid, 50 scid/+, and 34 C·B-17 (+/+) mice. Tissues were fixed in Carson's 10% neutral formol,[12] embedded in methacrylate glycol (JB-4, Polysciences, Inc., Warrington, Pa), and sectioned at 2 μ on a Sorvall JB-4 microtome (DuPont Instruments, Newtown, Conn). They were stained routinely with hematoxylin and eosin, and on occasion with Giemsa, periodic acid–Schiff reagent, Gridley's silver for reticulin, and Masson's trichrome for connective tissue.

Peripheral blood was tallied in a Coulter counter, and differential counts were performed manually.

Results

Pathology

Peripheral Blood

Leukopenia was constant in scid mice because of a paucity of circulating lymphocytes (Table 1). The total leukocyte count among 34 scid mice varied from 1500 to 4500/cu mm, whereas control mice varied between 4500 and 12,000/cu mm. The small proportion of lymphocytes in scid mice (14–31%) was contrasted by a relative granulocytosis of 66–81%. Conversely, in control mice, lymphocytes ranged between 78% and 97%, and granulocytes comprised a minor fraction of the total leukocytes (3–22%). Hematocrits were relatively constant in both scid and control mice, varying between 45% and 50%. However, we suspect compensated hemolytic anemia, because many scid mice showed marked erythropoietic hyperplasia in their spleens, as well as hemosiderosis.

Thymus

Scid thymus glands were very small, usually comprising several lobules ranging from 1 × 2 to 2 × 5 mm, grouped or widely scattered in retrosternal fibroadipose

Table 1—Range of Peripheral Blood Leukocyte and Differential (100-Cell) Counts in Scid and Control Mice

Mouse†	No. of mice	Total leukocytes (cells/cu mm)	Neutrophils (%)	Eosinophil (%)	Lymphocytes (%)	Monocytes (%)
scid	21*	1,500–4,500	66–81	0	14–31	2–4
scid/+	13	4,500–9,700	3–21	0–1	78–97	1–7
C.B-17 (+/+)	14	4,300–10,900	2–18	0	78–90	1–4
BALB/c	9	9,400–12,000	6–22	0–2	78–93	0–1

* Not including 13 mice with total leukocyte counts between 1,500 and 2,000/cu mm and too few cells in smears for adequate differential counts.
† Hematocrits were remarkably constant in all four strains, ranging between 45% and 50%; on scan of the smears, platelets also fell within the rather broad range of normal.

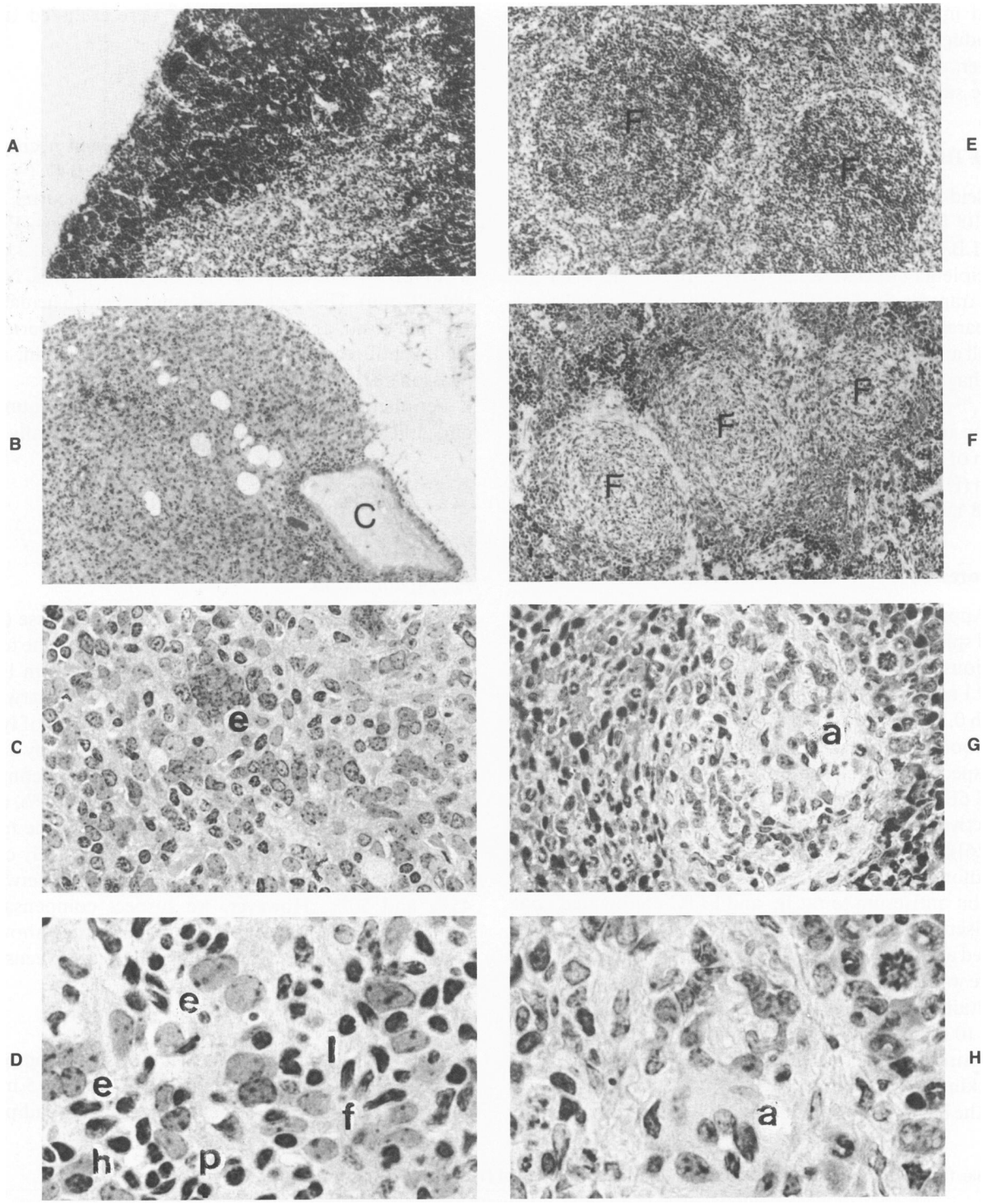
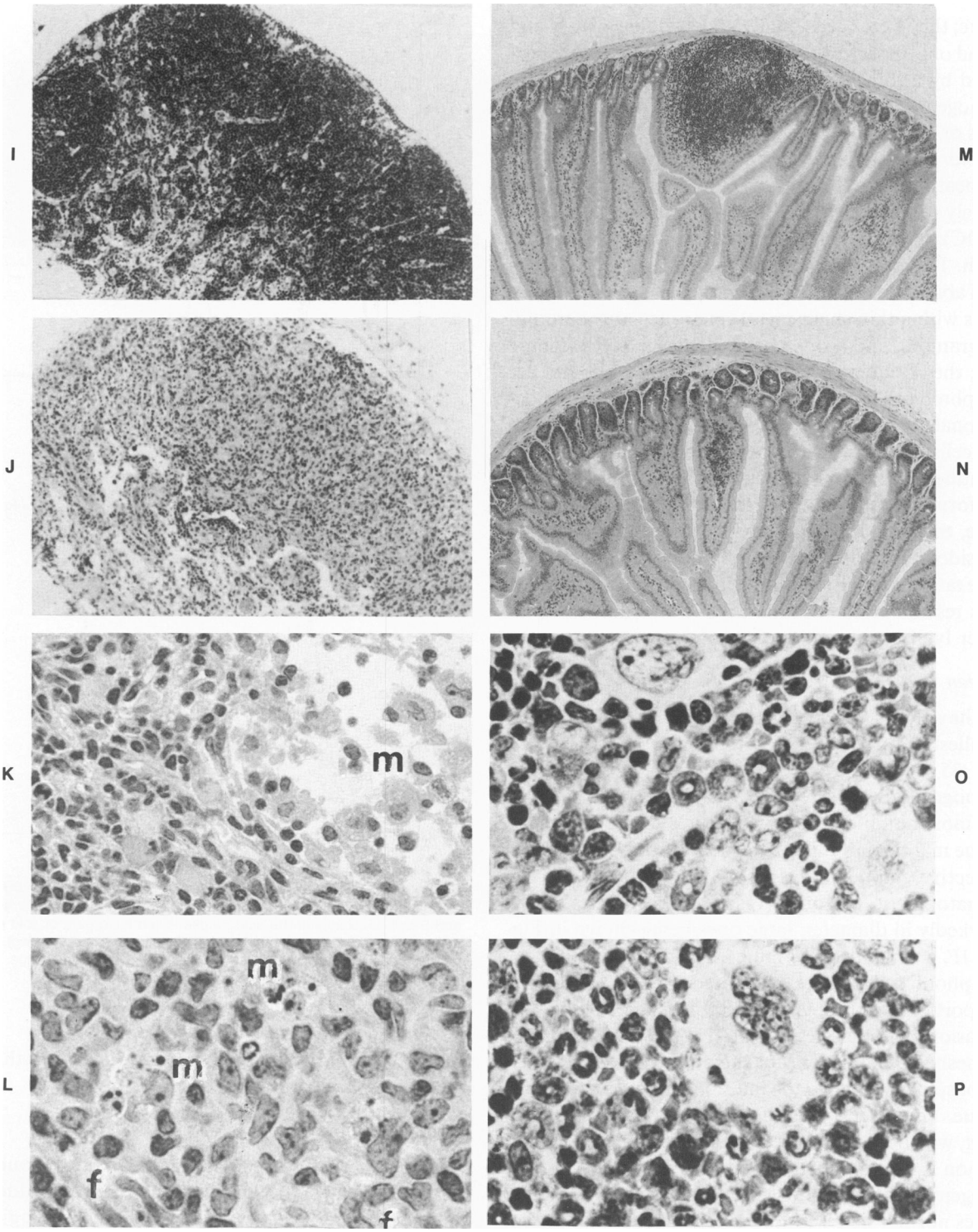


Figure 1—Comparative histology of scid and scid/+ mice. **A**—Scid/+ thymus. Appears normal, with a dense lymphocytic cortex and sharply defined corticomedullary junction. (×80) **B**—Scid thymus. Completely devoid of cortex, resembling the scid/+ medulla. Cysts (*C*) are common, as well as small aggregates of ft. (×80) **C**—Scid thymus. Parenchyma formed by acidophilic hyaline stroma loosely sprinkled with several cell types in varying proportions, epithelial components often forming tufts (*e*). (×400) **D**—Scid thymus. Epithelial cells (*e*) are readily distinguished by their larger size and delicate nuclear quality; admixed lymphoid cells (*l*) are usually larger than adult lymphocytes, while histiocytes (*h*), fibroblasts (*f*) and rare plasmacytes (*p*) can be identified. (×800) **E**—Scid/+ spleen. Malpighian follicles (*F*) fully populated with small lymphocytes; Intervening pulp contains usual hematopoietic tissue in varying proportions. (×80) **F**—Scid spleen. Follicles (*F*) appear relatively empty. The pulp is filled with active erythroid, granulocytic, and megakaryocytic components in varying proportions comparable to scid/+ spleen. (×80) **G**—Scid spleen. A small follicle forms fibrous swirl around eccentric arteriole (*a*), devoid of lymphocytic sheath. (×400) **H**—Scid spleen. The periarteriolar zone of the follicle above



formed by loose connective tissue (containing a rich reticulin network, not shown) sprinkled with histiocytes and fibroblasts, a megakaryocyte in multipolar mitosis can be seen in the upper right portion. (×800) **I**—Scid/+ lymph node. Cortex, paracortical zone, and medullary cords are rich in lymphocytes. (×80) **J**—Scid lymph node. Retains fibrous framework and sinus pattern but is poorly populated. (×80) **K**—Scid lymph node. Outer cortex with marginal sinus containing many macrophages (*m*). The field is unusually rich in lymphocytes. (×400) **L**—Scid lymph node. Contrast area in paracortex with predominance of macrophages (*m*, with engulfed debris) and fibroblasts. (×800) **M**—Scid/+ intestine. Well-developed lymphoid follicle in submucosa. (×60) **N**—Scid intestine. Follicular sites are poorly populated. (×60) **O**—Scid/+ bone marrow. Predominance of granulocytes with intermingled red cell precursors and varying numbers of megakaryocytes. (×650) **P**—Scid bone marrow. No significant difference from scid/+ on light microscopy. (×650) Difference demonstrated on FACS profiles (Figure 4). (Panels A, B, E, F, I, J, O, P reprinted by permission from Nature 1983, 301[5900]: 1–4, courtesy of Macmillan Journals, Ltd.)

tissue; they were occasionally undetectable grossly and found only in sections of the thymic site. They were bordered by a thin fibrous capsule enclosing a relatively homogeneous parenchyma, sometimes containing small cysts or clusters of fat cells (Figure 1B) and completely devoid of a lymphocytic cortex or corticomedullary delineation. A substantial fibrocollagenous stroma was loosely sprinkled with cells of varying appearance (Figure 1C); reticulin fibrils formed a delicate background mesh. The epithelial components were large ovoid cells with abundant but ill-defined cytoplasm, a vesicular nucleus with a thin sharp membrane, a few tiny chromatin granules, and a small prominent nucleolus (Figure 1D); they frequently grew as dense tufts. The smaller lymphoid cells with hyperchromatic nuclei and an occasional nucleolus varied in size, while stellate and spindle cells making up the remaining population were identified as macrophages and fibroblasts, respectively, the former often containing nuclear debris. Among the mice, the proportions of the several cell types varied considerably. Lymphatic and blood vascular channels were rather sparsely distributed. In general, the scid thymus resembled the normal thymic medulla, but with fewer lymphocytic components.

Spleen

The spleens of scid mice varied greatly in size, the smallest measuring 1 × 2 × 10 mm, the largest 5 × 8 × 35 mm; follicles were never grossly visible in the homogeneous dark red to reddish gray pulp. One of the most notable histologic features of the spleen lay in the malpighian follicles, which, through a scanning objective, appeared empty in contrast to the hematopoietic red pulp (Figure 1F). Follicles varied markedly in diameter, large ones being shown in Figure 1F, a small one in Figure 1G. They contained few lymphoid cells, being populated largely by varying proportions of fibroblasts and macrophages along with occasional plasmacytes (Figure 1H). Fine reticulin fibrils enmeshed the follicles (not shown), whereas they were inconspicuous among the interfollicular hematopoietic tissue. The latter represented a highly reactive component, which is hardly surprising, since myeloid differentiation is not impaired in scid mice.[5,6] In the event of pyogenic infections, the spleen was often two to three times normal size, with the pulp converted almost entirely to granulocytes of striking immaturity, usually associated with marked megakaryocytosis; the defective follicles were sometimes obscured. Scid mice with presumed anemia presented large spleens almost completely occupied by nucleated red cells alone or associated with granulopoiesis and/or megakaryocytosis. Intravenous injection of particulate matter disclosed

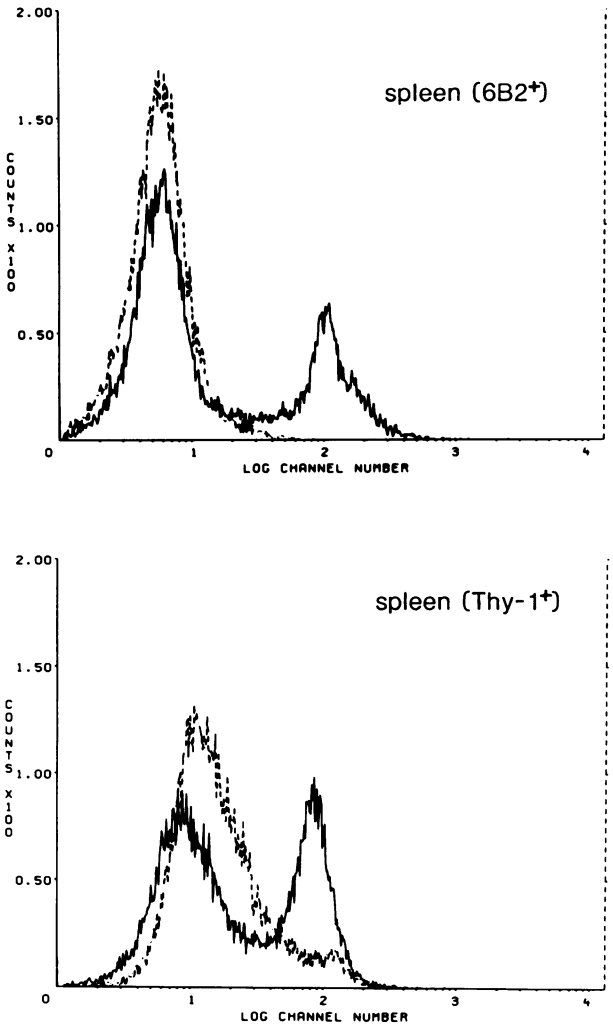
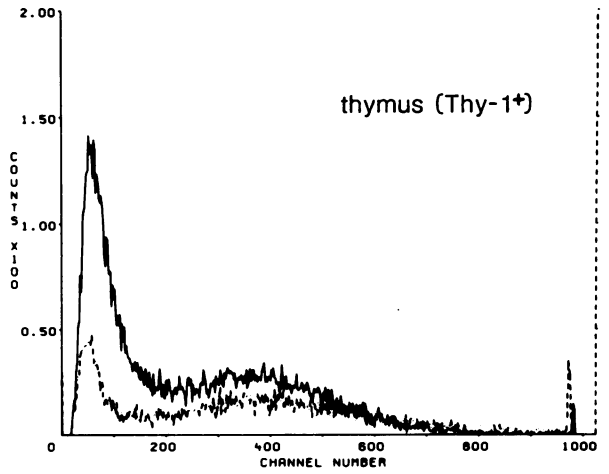


Figure 2—FACS analysis for Thy-1.2⁺ and 6B2⁺ (positive with 6B2 anti-Ly-5 [B220]) cells in spleens of scid and scid/+ mice, demonstrating T- and B-cell deficiency in the scid mouse. Each histogram of scid mouse (*dotted tracing*) is superimposed on that of the scid/+ (*solid tracing*). Channel numbers less than 1.5 correspond to background fluorescence. (x axis = log intensity of fluorescence; y axis = number of cells)

cells of monocyte/macrophage type, richly in the red pulp, sparsely within the follicles.

Lymph Nodes

Nodes were tiny in scid mice and generally found in their usual sites as firm shotty nodules embedded in adipose tissue. Most were identifiable histologically through retention of their basic stromal structure and sinus pattern (Figure 1J). Their population varied greatly in number and proportion of cell types (Figure 1K and L). Lymphoid cells were generally sparse, with macrophages usually prominent in sinuses and medullary cords, while fibroblasts were often active and occasionally converted the node into a fibrocollagenous



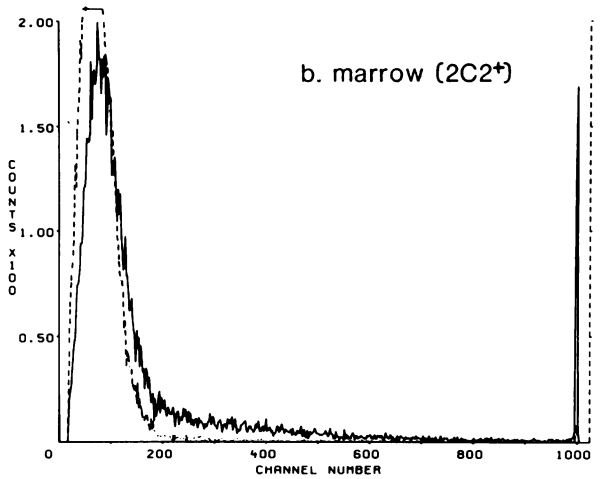
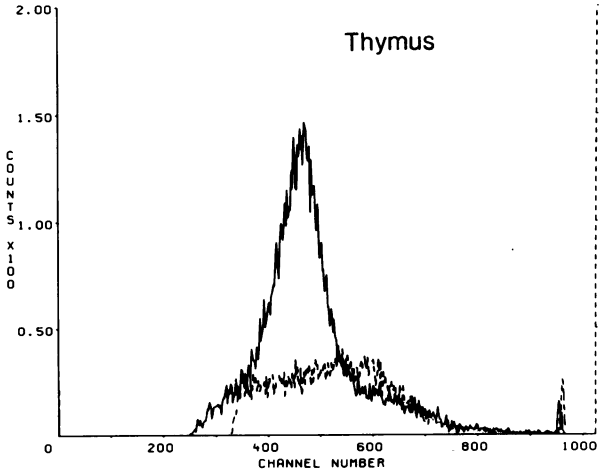


Figure 3—FACS analysis for Thy-1.2+ cells in thymus and 2C2+ (positive with 2C2 anti-Ly-5 [B220] cells in bone marrow of scid and scid/+ mice, furnishing evidence for T cells but not for pre-B cells in the scid mouse. Each histogram of scid mouse (*dotted tracing*) is superimposed on that of the scid/+ (*solid tracing*). Channel numbers less than 200 indicate background. (x = intensity of fluorescence; y axis = number of cells)



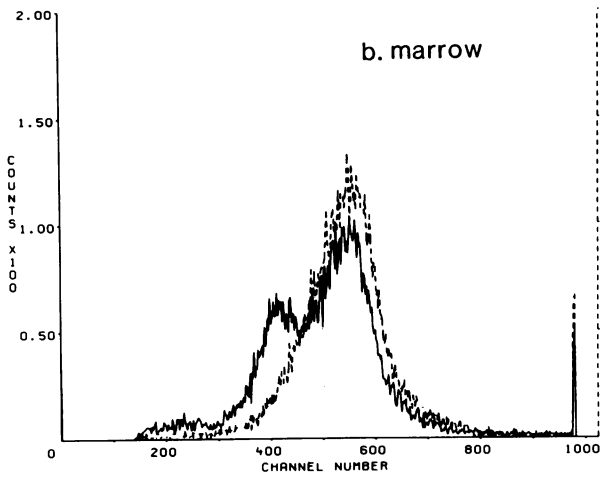


Figure 4—Comparison of FACS cell size profiles for thymus and bone marrow, respectively, of scid and scid/+ mice, indicating a disproportionate loss of small cell populations in the scid mouse. Each histogram of scid mouse (*dotted tracing*) is superimposed on that of the scid/+ (*solid tracing*). (x axis = forward light scatter; y axis = number of cells)

nodule. In a few instances plasmacytosis was prominent, and in two cases, even Russell bodies were found. Reticulin fibrils were sparse and ill defined.

Bone Marrow

Sections of marrow from scid mice were not detectably different from those of control mice (Figure 1O and P). In each, granulocytes predominated, with clusters of erythrocytic precursors scattered irregularly and megakaryocytes varying from few to many. A relative increase in erythroid components usually accompanied splenic erythropoietic hyperactivity. Neither scid nor control mice showed the lymphocytic aggregates frequently seen in human marrow.

Other Lymphatic Sites

Peyer's patches and solitary follicles of the intestinal tract were practically nonexistent in scid mice (Figure 1N), as were submucosal lymphoid aggregates in the tracheobronchial tree.

FACS Analysis

Lymphoid tissues of more than 30 scid mice were analyzed with an FACS, along with counterparts from scid/+ mice, with the use of different monoclonal antibodies reactive with pre-B cells and B cells or T cells. Cells of the B lineage were not detectable in spleen or

marrow. However, putative T cells were routinely found; ie, in most scid mice ⩾ 60% of thymocytes and 5–25% of splenocytes were Thy-1.2⁺. As the thymus and spleen of scid mice generally yielded less than 0.7×10^6 and 20×10^6, respectively, these percentages actually represent relatively few T cells. Moreover, such T cells are presumably nonfunctional, as no T-cell activity has as yet been demonstrated in scid mice.[5,6] Some of the above FACS results were summarized in our earlier publication.[5]

Here we show representative FACS profiles of the lymphocytic deficiency in thymic, splenic, and marrow cells of scid mice (Figures 2 and 3). Histograms of the intensity of fluorescence for scid and scid/+ cells are superimposed. Few Thy-1.2⁺ and no B cells are detected in scid splenocytes (Figure 2). Moreover, scid bone marrow lacks detectable pre-B cells (Figure 3). Thymocytes of both scid and scid/+ show an equally broad distribution for the density of Thy-1.2 determinants (Figure 3).

FACS analysis also clearly demonstrated striking differences in the size distributions of cells from lymphoid tissues of scid and scid/+ mice. Representative histograms of cell number versus cell size are shown in Figure 4. Histograms for scid thymocytes were relatively broad; they lacked the uniform, sharp peak characteristic of those from scid/+ mice. Clearly, large thymocytes comprised a greater proportion of the total thymic population in scid than in scid/+ mice. With respect to bone marrow, cell size distributions were unimodal for scid and bimodal for scid/+ mice. Apparently, one or more distinct population(s) of small cells present in the bone marrow of scid/+ mice is missing in scid mice.

Reconstitution

Eight scid mice which had been injected 5–10 months earlier with histocompatible bone marrow cells of C·B-17, BALB/c, or (B6·C-9 × BALB/c)F_1 mice were examined for lymphoid reconstitution. Two were fully reconstituted and were indistinguishable from control C·B-17 mice. Reconstitution was variable and incomplete in the remaining six mice (Table 2). Neoplasia did not occur in any. Neither the source or numbers of injected cells nor the elapsed time between graft and necropsy appeared to influence the result. Specifically, the tissue changes were as follows.

Thymus

In the two fully reconstituted scid mice, the cortex was of normal breadth and penetrated by trabeculate incursions of the fibrous capsule, creating a scalloped margin. The corticomedullary border was distinct (Figure 5A). Maturation of lymphocytes within the cortex was clearly defined; in the immediate subcapsular zone they were large, with vesicular nuclei and prominent nucleoli, frequently mitotic and loosely arranged (Figure 5B). Cells of the cortical midzone were smaller, rounded, uniform in size and closely packed (Figure 5C). Toward the medulla they were still smaller and tightly arranged (Figure 5D). From here the cortical lymphocytes appeared to migrate among the medullary epithelial and supportive components and could be found within the lumens of blood and lymph channels where they appeared fully mature (Figures 5E and F). Carbon particles were injected intravenously into 6 reconstituted scid mice and 3 scid/+ controls to evaluate the "blood–thymus barrier."[8] Particles were found in macrophages of the medulla, but not in the cortex.

Lymph Nodes

The cortex and paracortex of the nodes were completely restored, germinal centers appearing in the nodal cortex in 2 mice (Figure 5G). In a node of 1 scid mouse about half of the parenchyma was densely lymphocytic, while the remainder had been converted into pure bone marrow (Figure 5H).

Table 2—Reconstitution of Scid Mice by Intravenous Transfer of Histocompatible Bone Marrow

Donor	No. of cells	Graft to necropsy (weeks)	Lymphocytosis				
			Thymus	Spleen	Nodes	Gut	Lung
BALB/c	5×10^6	24	4+	4+	4+*	4+	4+
C.B-17	3×10^6	42	4+	4+	4+	4+	2+
BALB/c	1.5×10^6	40	3+	3+	†	2+	1+
C.B.-17	3×10^6	23	3+	3+	3+*	4+	1+
C.B.-17	3×10^6	23	2+	3+	2+	2+	0
C.B.-17	3×10^6	40	1+	1+	3+	‡	1+
(B6.C9 × BALB/c)F_1	3×10^6	40	1+	2+	1+	‡	1+
BALB/c	3×10^6	42	1+	1+	1+	‡	‡

* Nodal cortices contained germinal centers.
† Nodes grossly enlarged; no sections.
‡ No sections.

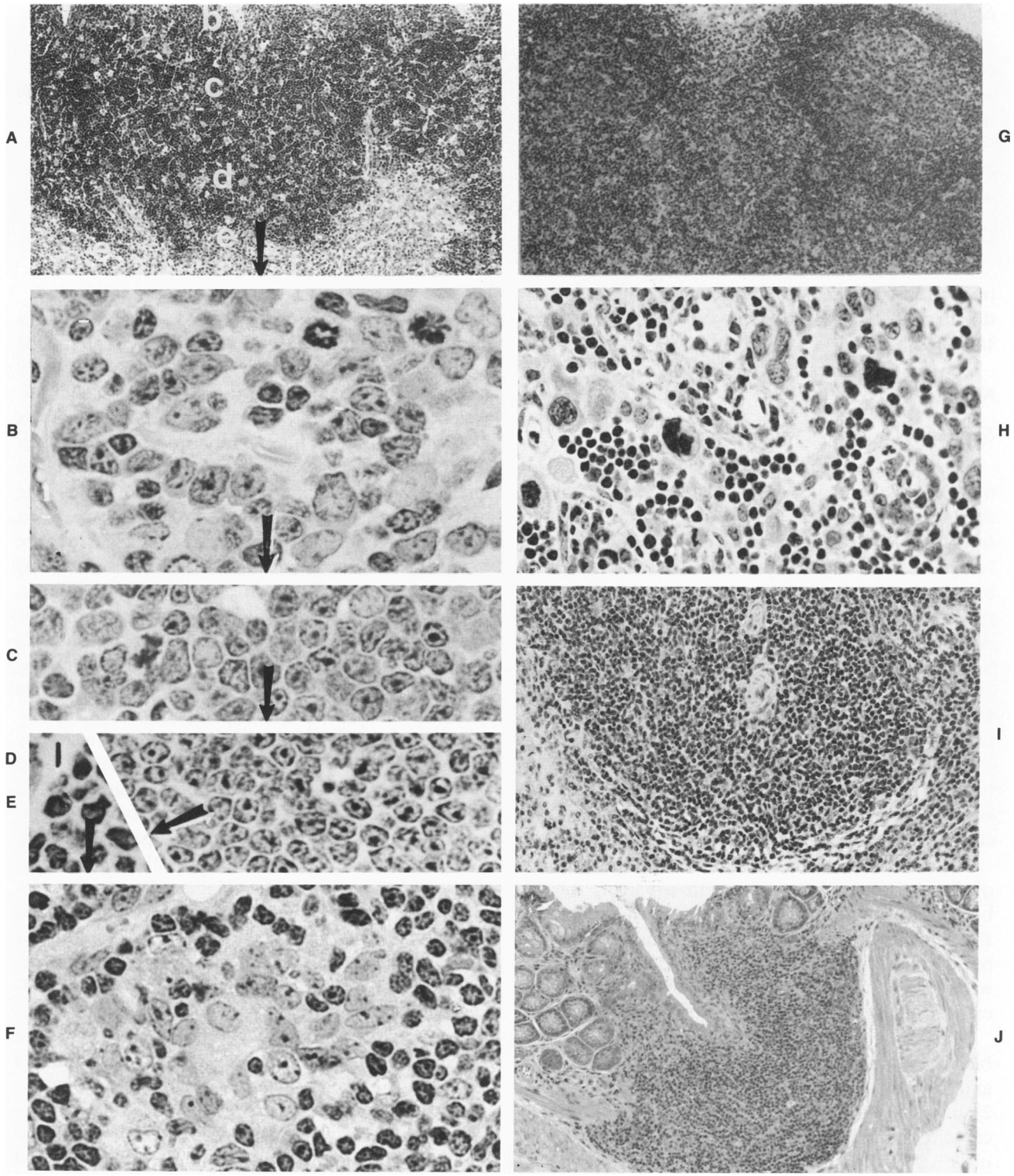


Figure 5—Reconstitution of scid mouse by intravenous injection of bone marrow suspension (3×10^6) from conventional C.B-17 mouse (graft to necropsy, 42 weeks). **A**—Thymus. Normally structured with clearly marginated corticomedullary junction (×60). (Lowercase white letters indicate sites of panels **B**, **C**, **D**, **E**, and **F** below). **B**—Thymus. Subcapsular cortex with loosely arranged large lymphoid cells, mitotically active and appearing immature (pre-T cells). (×800) **C**—Thymus. Midcortex with smaller and more uniform lymphoid cells closely arranged. (×800) **D**—Thymus. Inner cortex with still smaller and more evidently mature cells. (×800) **E**—Thymus. Lymphatic channel (*1*) in immediately subjacent medulla distended with virtually mature lymphocytes. (×800) **F**—Thymus. Medulla populated by an admixture of epithelial cells and lymphocytes. (×800) **G**—Lymph node. B and T-cell zones fully populated, with germinal centers evident in cortex. (×100) **H**—Lymph node. Half of a node in this mouse was populated with lymphocytes as in panel **G**; the other half (*shown here*) was converted to bone marrow with full complement of erythroid, granulocytic and megakaryocytic components. (×400) **I**—Spleen. A malpighian follicle appears normal. (×250) **J**—Cecum. Large solitary lymphoid follicle restored, along with Peyer's patches in duodenum (not shown). (×100)

Spleen

Follicles of the spleen contained a normal lymphocytic population, appearing to emanate from the periarteriolar sheath and often pushing the original fibroblastic component into a pseudocapsular margin (Figure 5I). The new follicles varied considerably in size, and faint outlines of germinal centers could occasionally be distinguished.

Other Lymphatic Sites

Peyer's patches and solitary follicles of the intestinal tract returned more or less completely (Figure 5J), as did the small lymphocytic aggregates in the respiratory submucosa.

Neoplasia

Spontaneous T-Cell Lymphomas

The incidence of a nonleukemic, poorly differentiated malignant lymphoma was strikingly high in scid mice. As shown in Table 2, spontaneous lymphomas were detected in 41 of 269 necropsied mice (no such tumors have been observed in normal C·B-17 mice). Lymphomas were found in scid mice of all ages, with males appearing slightly more disposed than females (ratio, 3:2). All scid lymphomas tested thus far by FACS analysis have been typed as Thy-1.2$^+$ (data summarized in Table 3). Three lymphomas examined for Ly-1 and Ly-2 were found to be positive for both markers. None was found to express B-cell markers (Ly-5 [B220]).

The spontaneous tumors were transmissible to histocompatible recipients by injection of cultured cell suspensions or direct implants of fragments; we have carried them through five generations.

Scid lymphomas formed diffuse homogeneous sheets of fairly uniform blast cells with a narrow rim of basophilic cytoplasm and a vesicular nucleus having a sharp membrane, fine to coarse chromatin granules, and one or more large nucleoli (Figure 6A); mitotic activity was marked. Stroma was absent except for a sparse delicate fibrillar skein that had no intimacy with the cells.

The thymic origin of the lymphomas was indisputable. Not only were tumors found solely within the lobes and lobules of the gland in 18 of the 41 cases (Table 2), but in 4 cases completely uninvolved lobes of typical scid thymus lay adjacent to neoplastic lobes (Figure 6B); further, in the latter, the tumor cells were slightly smaller and more compact than in the disseminating growths, and occasional residual epithelial cells could be distinguished in the background. Again, in 3 other cases the "ghost" outline of a thymic lobe was found within a locally invasive retrosternal mass (Figure 6C). Lung involvement took place along peribronchial and perivascular lymphatic channels (Figure 6D). Metastasis to mediastinal, cervical, and intraabdominal lymph nodes was seen in 1 case, while in 2 others nodal extension was supplemented by early neoplastic change in the previously "empty" splenic follicles (Figure 6E). In the other 17 cases the tumor was disseminated to the lung, lymph nodes, spleen, liver, and kidneys, occasionally involving the genitalia of females. The splenic pattern was rather consistent, the tumor seeming to emanate from follicles, spreading through the red pulp to coalesce and replace the entire parenchyma. Extension to the liver likewise followed a uniform course, both with spontaneous and transplanted tumors, to form dense periportal aggregates with extensive filamentous permeation of sinusoids throughout the lobules (Figure 6F). The bone marrow was involved in only 1 case, and even here considerable amount of hematopoietic tissue persisted.

Table 3—Detection of Spontaneous Lymphomas in 41 of 269 Scid Mice That Underwent Necropsy

Extent of tumor	Number of mice		Age (weeks)
	Male	Female	
Thymus only	13	5	10–65
Locally invasive	1	2	16–60
Thymus + nodes	1	0	34
Thymus + nodes + splenic follicles	2	0	32–56
Disseminated	8	9	20–60
Total	25	16	

One rather bizarre case was encountered, a spongy thymic tumor 5 mm in diameter in a 52-week-old scid mouse with lymphnodal and splenic enlargement. The thymic tumor formed a multitude of intercommunicating capillary to cavernous spaces (Figure 7A) lined, respectively, by delicate spindle cells with nuclei of varying size and hyperchromatism (Figure 7B) to bulky pleomorphic, frequently multinucleated forms that tended to exfoliate into the lumens (Figure 7C). The spleen was large by virtue of hematopoietic hyperplasia, but the widely spaced follicles were converted to the typical T-cell lymphoma previously described. The lymph nodes were populated by densely packed round cells (Figure 7D) with vesicular nuclei having a thin sharp membrane and one or two prominent nucleoli; mitoses were frequent (Figure 7E). The tumor cells resembled histiocytes rather than those of the conventional T-cell type found in the spleen (Figures 7F and G). This may well be an example of multiple phenotypic expressions of lymphoreticular neoplasia as encountered in humans.[13]

Nonlymphomatous Tumors

Only four neoplasms other than the T-cell lymphomas were encountered in 269 scid mice that underwent necropsy. Two were papillary adenocarcinomas of the

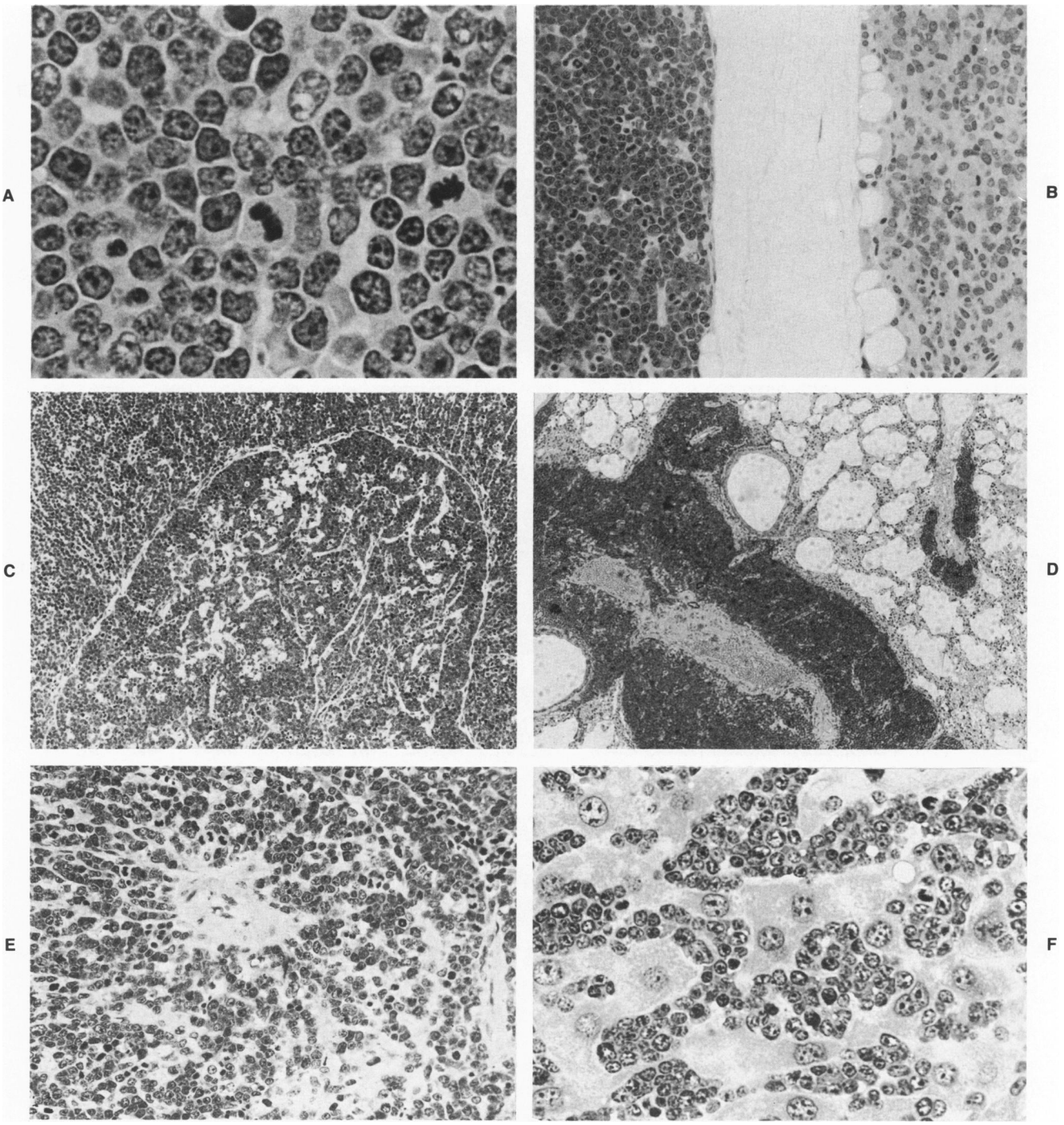


Figure 6—The T-cell thymic lymphoma of scid mice. **A**—Homogeneous sheet of large lymphoblastic cells with a narrow rim of basophilic cytoplasm and a coarsely hyperchromatic nucleus containing one or more nucleoli; mitoses are numerous. Stroma is limited to a few reticulin fibrils (not shown) unassociated with individual cells. (×1250) **B**—Two adjacent thymic lobes, the right a typical scid thymus, the left an early conversion to tumor (no tumor found elsewhere). (×250) **C**—Thymic "ghost" in retrosternal mass. Tumor cells within capsule are smaller and more tightly packed than in freely growing invasive perimeter (no tumor found elsewhere). (×100) **D**—Lymphatic permeation of lung is constant feature of spreading tumor. (×65) **E**—Splenic involvement begins in previously "empty" malpighian follicles which later coalesce to occupy the entire organ. (×250) **F**—Sinusoids of affected liver are characteristically permeated by cords of tumor stemming from periportal foci (not shown). (×400)

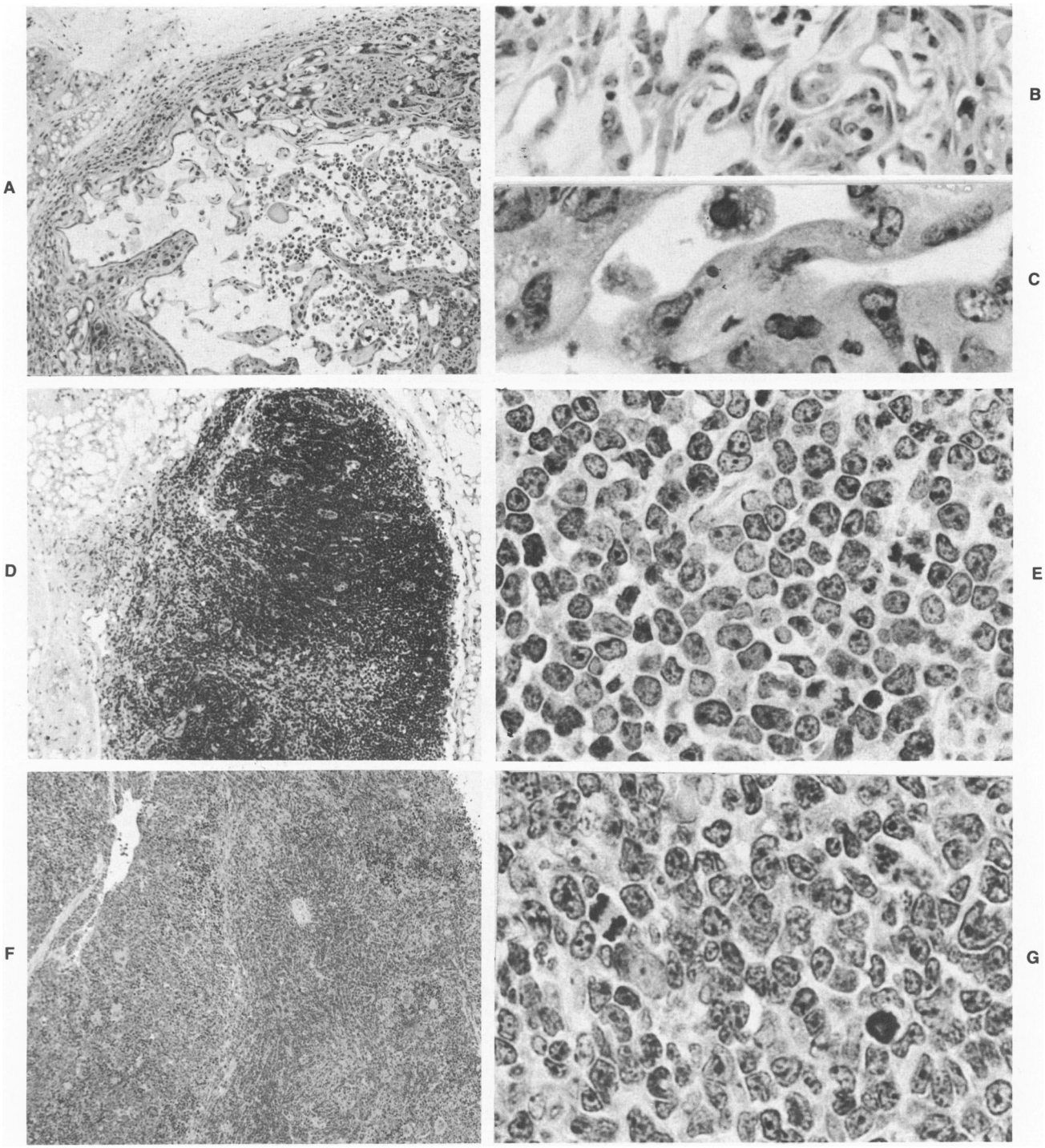


Figure 7—Composite neoplasia in an scid mouse. **A**—Thymus. Tumor composed of capillary to cavernous vessels lined respectively by delicate spindled cells (**B**) and large bizarre cells (**C**), classified as a lymphangioendothelioma. (**A** ×80; **B** and **C**, × 1250) **D**—Lymph node. All nodes examined are regarded as histiocytic lymphoma. (× 100) **E**—Nodal tumors show cytologic variance from scid T-cell lymphoma by virtue of smooth delicate nuclei. (× 800) **F**—Spleen with follicles replaced by lymphoma. (× 100) **G**—Cells of splenic tumor conform morphologically to those of classic scid T-cell lymphoma, nuclei being coarsely hyperchromatic. (× 800)

lung, one well differentiated, the other poorly so, a type of tumor commonly found in BALB/c and closely related strains. The other two tumors were adenocarcinomas, one arising in the breast, the other in the endometrium.

Discussion

Pathology

The histologic abnormalities in scid mice were strikingly uniform, consistent with the fact that all scid mice share the same genetic defect. Lymphopenia, a rudimentary thymic medulla, relatively empty splenic follicles and lymph nodes, and undeveloped bronchial and gastrointestinal lymphoctyic foci were characteristic. The lymphoid cells sparsely scattered in these sites varied in size, often appearing immature. FACS studies revealed T cells in thymus and spleen. Conventional pre-B and B cells could not be identified in any tissue examined, although a few mice showed variable numbers of plasma cells which correlated well with the detection of serum immunoglobulin (unpublished results). Except for the apparent deletion of one or more small cell population(s) (Figure 4), the bone marrow was morphologically indistinguishable from that of normal mice; cells of the myeloid lineage appeared normal and remained reactive throughout life. Concomitant lesions were generally related to infection or neoplasia.

Human patients with SCID also show lymphopenia and severe hypoplasia or dysplasia of lymphoid tissues.[1,2,14-18] Although different patterns of thymic dysplasia,[19,20] variable numbers of B lymphocytes,[20,21] and absence or presence of serum immunoglobulin[14-18,22,23] have been noted from case to case, such differences are not surprising, because the human SCID syndrome is known to result from different genetic lesions.[2,24,25]

Because the histologic abnormalities of SCID are so similar in both species, the scid mouse would seem to be a simple and useful model for human SCID. The only other animal model for SCID of which we are aware was reported by McGuire et al in Arabian foals.[26] Affected foals show a lymphocytic deficit in thymus, lymph nodes, and spleen and die of infection within a few months after birth. SCID infants suffer the same fate and invariably die before 2 years of life. Most scid mice ($\geq$ 60%), on the contrary, by virtue of their SPF environment, show a life span of 40–80 weeks (unpublished data), which is about three-fourths of the normal.

Reconstitution

In earlier reports we showed that scid mice could be reconsituted with lymphocytes derived from normal syngeneic bone marrow,[5] and that both T- and B-cell function could be demonstrated in reconstituted scid mice.[6] As indicated in this study, thymic and extrathymic lymphoid sites were partially to completely restored in reconstituted scid mice. Some tissues may have been "seeded" with only one or two progenitor cells already committed to either the myeloid or the lymphoid pathway.[27] This might explain the striking dichotomy in one reconstituted lymph node (Figures 5G and H), half of which appeared as bone marrow the other as a normal lymph node.

Because thymic dysplasia is the pathologic hallmark of SCID, the reconstitution of the scid thymus is of particular interest. Whereas the scid thymus consisted of only a rudimentary medulla, the reconstituted thymus showed a prominent lymphoid cortex and corticomedullary junction. Proceeding from capsule to medulla, the character and arrangement of lymphoid cells changed from immature, mitotically active, and loosely arranged to more mature, uniform, and closely arranged cells (Figure 5). Such a morphologic gradient is consistent with previous descriptions of the maturation process of thymic T cells[28,31] We conclude the scid thymus is a normal developmental structure which provides an adequate environment for thymocytic maturation but lacks functional T-cell precursors.

The so-called "blood–thymus barrier" allows blood-borne macromolecules to reach the medulla but prevents them from leaving the cortical capillary network. This barrier consists of a tight endothelial-macrophage system demonstrated by the elegant electron-microscopic studies of Raviola and Karnovsky[8] in the rat. Restoration of this barrier in the reconstituted scid thymus was indicated by demonstration of injected carbon particles in macrophages of the medulla and their absence in the newly formed cortex.

Neoplasia

The incidence of lymphoma in scid mice was strikingly high, occurring in 41 of 269 animals that underwent necropsy (15%) as opposed to 4 tumors of other types, all epithelial (1.5%). The lymphomas arose in the thymus, and the early intrathymic tumors appeared at all ages (10–65 weeks) (Table 3). Eight tumors were serotyped, all being identified as T-cell lymphomas (Table 4); the morphologic characters of all 41 were identical. Because T-cell lymphomas are rarely, if ever, observed in normal C·B-17 and BALB/c mice, their frequent occurrence in scid mice must be a direct or indirect consequence of the *scid* mutation.

Contrasting with the above, SCID infants and children seem disposed to develop B-cell lymphomas,[32] especially after treatment with thymic factors or grafts[33]

Table 4—Serotype Analysis of Spontaneous scid Lymphomas

	B-cell markers			T-cell markers		
Tumor	Ig	Lyb 8.2	Ly-5 (B220)	Thy-1.2	Ly-1	Ly-2
12	–	–	–	+	+	+
21	–			+		
28	–			+		
56	–	–	–	+	+	+
74	–	–		+		
366	–	–		+		
825	–			+		
1017	–			+	+	+

(interestingly, no lymphomas have been reported in SCID infants implanted with normal bone marrow[34]). These B-cell lymphomas in SCID infants may result from Epstein-Barr virus (EBV) infection (personal communication, Dr. Fred Rosen), as appears to be the case in patients with so-called X-linked lymphoproliferative syndrome. The latter syndrome is characterized by acquired agammaglobulinemia, serious or fatal infectious mononucleosis, or malignant B-cell lymphoma.[35,36] Affected patients are presumably unable to mount a specific immune response to EBV.

It is important to note that in some immune disorders, viruses may be responsible for both the lymphoid deficiency and malignancy. For example, infection of cats with feline leukemia retrovirus can lead to both immunosuppression and T-cell leukemia.[37,38] The HTLV retroviruses may cause similar effects in humans.[39,40] HTLV III, which has been implicated as the primary cause of AIDS,[39-41] is cytopathic for helper T cells and is presumably responsible for the depletion of these cells.[3] It may also be responsible for the lymphoreticular lymphomatous variant, Kaposi's sarcoma, which develops in over one-third of AIDS patients, along with occasional conventional lymphomas.[43]

Whether an oncogenic virus or some other transforming genetic element is responsible for the T-cell lymphomas in scid mice remains to be determined. Clearly, thymic T cells are susceptible to neoplasia as a result of the germline *scid* mutation. But one or more oncogenic events in the genome of scid T cells may also be required which, in the absence of any immune regulation or suppression, lead to T-cell malignancy.

References

1. World Health Organization Scientific Group on Immunodeficiency, Geneva, Switzerland. Clin Immunol Immunopathol 1979, 13:296-359
2. Rosen FS, Cooper MD, Wedgwood RJP: The primary immunodeficiencies (review). N Engl J Med 1984, 311:235-242, 300-310
3. Siegal FP: Immune function and dysfunction in AIDS. Semin Oncol 1984, 11:29-39
4. Penn I: Depressed immunity and the development of cancer (review). Clin Exp Immunol 1981, 46:459-474
5. Bosma GC, Custer RP, Bosma MJ: A severe combined immunodeficiency mutation in the mouse. Nature 1983, 301:527-530
6. Dorshkind K, Keller GM, Phillips RA, Miller RG, Bosma GC, O'Toole M, Bosma MJ: Functional status of cells from lymphoid and myeloid tissues in mice with severe combined immunodeficiency disease. J Immunol 1984, 132:1804-1808
7. Bosma MJ, Bosma GC, Owen JL: Prevention of immunoglobulin production by allotype-dependent T cells. Eur J Immunol 1978, 8:562-568
8. Raviola E, Karnovsky MJ: Evidence for a blood-thymus barrier using electron-opaque tracers. J Exp Med 1972, 136:466-498
9. Coffman B, Weissman IL: A monoclonal antibody that recognizes B cells and B cell precursors in mice. J Exp Med 1981, 153:269-279
10. Coffman B, Weismann IL: B 220: A B cell-specific member of the T200 glycoprotein family. Nature 1981, 289:681-689
11. Ledbetter JA, Herzenberg LA: Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol Rev 1979, 47:63-90
12. Carson FL, Martin JH, Lynn JA: Formalin fixation for electron microscopy: A re-evaluation. Am J Clin Pathol 1973, 59:365-373
13. van den Tweel JG, Lukes RJ, Taylor CR: Pathophysiology of lymphocyte transformation: A study of so-called composite lymphomas. Am J Clin Pathol 1979, 71:509-520
14. Glanzman E, Riniker P: Essentielle lymphocytophthise: Ein neues Krankheitsbild aus der Sauglingspathologie. Ann Paediatr (Basel) 1950, 175:1-32
15. Tobler R, Cottier H: Familiare Lymphopenie mit Agammaglobulinämia und schwerer Moniliasis: Die essentielle Lymphocytophtise als besondere Form der fruhkindlichen Agammaglobulinamia. Helv Paediatr Acta 1958, 13:313-338
16. Hitzig WH, Biro Z, Bosch H, Huser HJ: Agammaglobulinamie und Alymphocytose mit Schwund de lymphatischen Gewebes. Helv Paediatr Acta 1958, 13:551-585
17. Breton A, Walbaum R, Boniface L, Gondemand M, Dupont A: Lymphocytophtisie avec dysgammaglobulinemie chez un nourrison. Arch Fr Pediatr 1963, 20:131-146
18. Nezelof C, Jammet MC, Lortholary P, Labrune B, Lamy M: L'hypoplasie hereditaire du thymus: Sa place et sa responsibilité dans une observation d'aplasie lymphocytaire normoplasmocytaire et normoglobulinemique du nourrisson. Arch Fr Pediat 1964, 21:897-920
19. Borzy MS, Schulte-Wissermann H, Gilbert E, Horowitz SD, Pellet J, Hong R: Thymic morphology in immunodeficiency diseases: Results of thymic biopsies. Clin Immunol Immunopathol 1979, 12:31-51
20. Gosseye S, Diebold N, Griscelli C, Nezelof C: Severe combined immunodeficiency disease: A pathological analysis of 26 cases. Clin Immunol Immunopathol 1983, 29:58-77
21. Cooper MD, Lawton AR: Circulating B cells in patients with immunodeficiency. Am J Pathol 1972, 69:513-528
22. Geha RS, Schneeberger E, Gatien J, Rosen FS, Merler E: Synthesis of an M component by circulating B lymphocytes in severe combined immunodeficiency. N Engl J Med 1974, 290:726-728
23. Hitzig WH, Landolt R, Müller G, Bodmer P: Heterogeneity of phenotypic expression in a family with Swiss-type agammaglobulinemia: Observations on the acquisition of agammaglobulinemia. J Pediatr 1971, 78:968-980

24. Hoyer JR, Cooper MD, Gabrielsen AE, Good RA: Lymphopenic forms of congenital immunologic deficiency diseases. Medicine (Baltimore) 1968, 47:201–226
25. Giblett ER, Anderson JE, Cohen F, Pollara BI, Meuwissen HJ: Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. Lancet 1972, 2:1067–1069
26. McGuire TC, Banks KL, Poppie MJ: Combined immunodeficiency in horses: Characterization of the lymphocyte defect. Clin Immunol Immunopathol 1975, 3:555–566
27. Abramson S, Miller RG, Phillips RA: The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. J Exp Med 1977, 145:1567–1579
28. Ritter MA: Embryonic mouse thymus development: Stem cell entry and differentiation. Immunology 1978, 34:69–75
29. Scollay R, Weissman IL: T cell maturation: Thymocytic and thymus migrant subpopulations defined with monoclonal antibodies to the antigens Lyt-1, Lyt-2, and ThB. J Immunol 1980, 124:2841–2844
30. Scollay R, Jacobs S, Jerabek L, Butcher E, Weissman I: T-cell maturation: Thymocytic and thymus migrant subpopulatons defined with monoclonal antibodies to MHC region antigens. J Immunol 1980, 124:2845–2853
31. Basch RS, Kadish JL: Hematopoietic thymocyte precursors: II. Properties of the precursors. J Exp Med 1977, 145:405–416
32. Kersey JH, Filipovich AH, Spector BD, Frizzera G: Lymphoma after thymus transplantation. N Engl J Med 1980, 302:301–302
33. Borzy MS, Hong R, Horowitz SD, Gilbert E, Kaufman D, DeMendonea W, Oxelius VA, Dictor M, Pachman L: Fatal lymphoma after transplantation of cultured thymus in children with combined immunodeficiency disease. N Engl J Med 1979, 301:565–568
34. Bortin MM, Rimm AA: Severe combined immunodeficiency disease: Characterization of the disease and results of transplantation. JAMA 1977, 238:591–600
35. Purtilo DT: Epstein-Barr-Virus-induced oncogenesis in immune-deficient individuals. Lancet 1980, 1:300–303
36. Purtilo DT, Szymanski I, Bhawan J, Yang JPS, Hutt LM, Boto W, DeNicola L, Maier R, Thorley-Lawson D: Epstein-Barr virus infections in the X-linked recessive lymphoproliferative syndrome. Lancet 1978, 1:798–801
37. Trainin Z, Wernicke D, Ungar-Waron H, Essex M: Suppression of the humoral antibody response in natural retrovirus infections. Science 1983, 220:858–859
38. Anderson LJ, Jarrett O, Laird HM: Feline leukemia-virus infection of kittens: Mortality associated with atrophy of the thymus and lymphoid depletion. J Natl Cancer Inst 1971, 47:807–813
39. Gallo RC, Sarin PS, Blattner WA, Wong-Staal F, Popovic M: T-cell malignancies and human T-cell leukemia virus. Semin Oncol 1984, 11:12–17
40. Sarngadharan MG, Popovic M, Bruch L, Schüpbach J, Gallo RC: Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 1984, 224:503–505
41. Popovic M, Sarngadharan MG, Read E, Gallo RC: Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and Pre-AIDS. Science 1984, 224:497–500
42. Schüpbach J, Popovic M, Gilden RV, Gonda MA, Sarngadharan MG, Gallo RC: Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 1984, 224:500–503
43. Reichert CM, O'Leary TJ, Levens DL. Simrell CR, Macher AM: Autopsy pathology in the acquired immune deficiency syndrome (review). Am J Path 1983, 112:357–382

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